DR. GEORG B. NEURATH Vertragsforschung und Beratung

THE COUNCIL FOR TOBACCO RESEARCH - U.S.A., INC.

110 East 59th Street New York, N.Y. 10022 2000 Hamburg 56 Hexentwiete 32 Telefon (040) 81 60 40 July 3, 1975

Plan of Work

NITROSAMINES IN TOBACCO AND ITS SMOKE OCCURRENCE, FORMATION, AND TRANSFER

Hoffmann, Hecht, Ornaf, and Wynder reported in Science (1974) on the occurrence of N-nitroso nornicotine (NNN) in unburned tobacco. The most striking value was reported in a brand of chewing tobacco (C; 88.6 ppm), i.e. 10-30-fold the concentration compared to all other tobacco samples analysed. The artefactual formation of NNN - considered to be conceivable by the authors - was obviously precluded only with two of the samples analysed in this study (cigarette B and chewing tobacco C).

Making use of the experiences collected in preceding studies on the behaviour of all reactants involved in the formation of nitrosamines and on analytical procedures, those results should be controlled.

Although the average content of nitrite in tobaccos is about 40 ppm (Lipp and Dölberg, 1964), the formation of nitrosamines in tobacco under certain conditions seems generally to be possible. The nitrite concentration could be that left after reaction with other tobacco constituents, i.e. also amines, under formation of nitrosamines.

Nitrite in tobacco is probably formed by reduction of nitrate, the concentration of which varies remarkably among tobacco varieties (Neurath and Ehmke, 1964; Lipp and Dölberg, 1964). The nitrite

1003540657

July 3, 1975

1003540658

contents of different tobacco products may also vary according to tobacco treatments and ageing processes. Technological steps could influence the formation of nitrosamines, as for instance in the case of chewing tobacco.

It should be studied how and how far preformed nitrosamines would be transferred from tobacco to main and side stream smoke.

Priority List

 Determination of volatile and non-volatile nitrosamines in a variety of tobacco products

Making use of the most dependable method - finally accepted method from the running studies - volatile and non-volatile nitrosamines - shall be determined in several cigarette, cigar, pipe, chewing tobaccos, and snuff.

Technological influences should be detectable by this survey.

necessary steps:

- 1.1 Comparison of different extraction methods for the nitrosamines as to artefactual formation. Possible influences can be due to solvent and $p_{\rm H}$ at extraction.
- 1.2 Comparison of different methods for gas chromatography detection with mass spectrometry. Chemiluminescence, electron capture, and N-specific detectors shall be compared to mass spectrometry as to specifity.

July 3, 1975

-3-

 Determination of volatile and non-volatile nitrosamines in tobacco varieties

The influence of tobacco growing conditions and fertilizers on the possible formation of nitrosamines in tobacco shall be studied under corresponding aspects to 1.

3. Formation of nitrosamines in tobacco

Facing the results of 1. and 2., it shall be studied, which of the tobacco constituents, and under which conditions and reactions lead to the formation of nitrosamines - nitrite or nitrate content, reduction of nitrate by tobacco constituents as for instance reducing sugars, katalytic effects, or special technological treatment of certain tobacco products, for instance chewing tobacco.

4. Transfer of nitrosamines from tobacco to smoke

Model studies on the transfer rates of volatile and non-volatile nitrosamines from tobacco to main and side stream smoke shall be accomplished by means of $^{14}\text{C-labelled}$ dimethylnitrosamine and N-nitroso nornicotine.

July 3, 1975

4

Anticipated duration

The duration of the study described is anticipated to be two to three years.

Estimated position - end of the 1 st year

The estimated position at the end of the first year is that determinations in tobacco products and in tobacco varieties will be completed.

Facilities

Gas chromatography and liquid chromatography of all kinds.

Chemiluminescence apparatus. Equipment for tracer studies available.

Administration

Dr. Georg B. Neurath, President

Staff

Four professionals, involved with microanalytical determinations of environmental chemicals, drug metabolism studies and other Trace substances.

Budget, 1 st year \$49,680 Projected, 2 nd year \$53,000

Proposed starting date: January 1, 1976